IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

I, ADRIAN PAUL BROWN, M.A., M.I.L., M.I.T.I., declare

1. That I am a citizen of the United Kingdom of Great Britain and Northern Ireland, residing at 5 Gilbert Road, London, SE11 4NZ.

2. That I am well acquainted with the German and English languages.

3. That the attached is a true translation into the English language of the Specification of International Patent Application No. PCT/EP2003/011603.

4. That all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the patent application in the United States of America or any patent issuing thereon.

DECLARED THIS 29th DAY OF APRIL 2005

A. P. BROWN

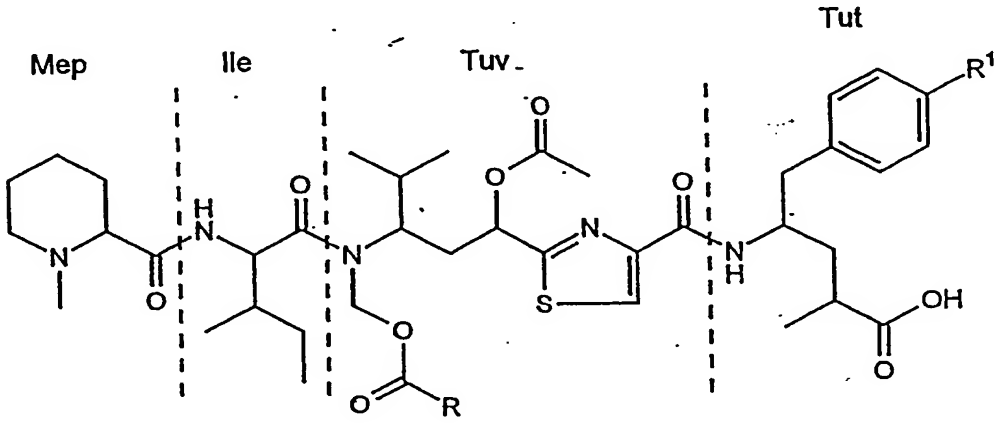
Our reference: 14431

New International Patent Application

Gesellschaft für Biotechnologische Forschung mbH (GBF)

Tubulysins, preparation processes and tubulysin preparations

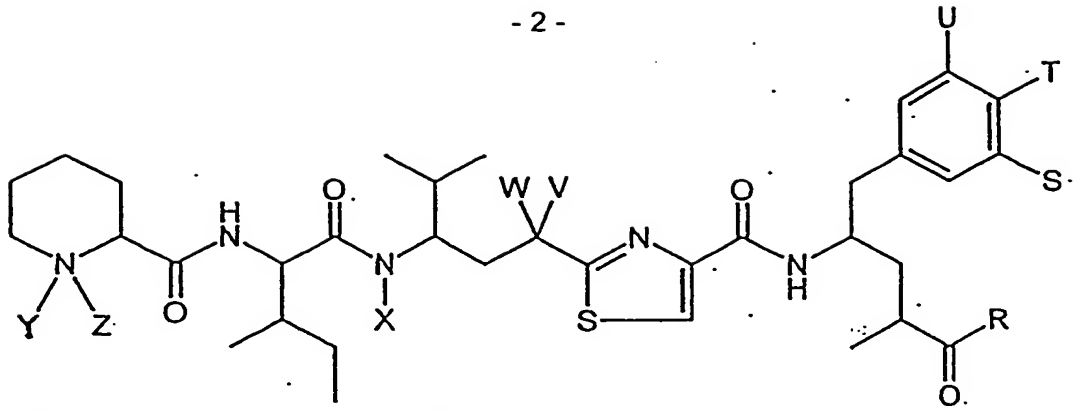
Tubulysins are known as compounds of the following general formula; cf., for example, F. Sasse, H. Steinmetz, J. Heil, G. Höfle, H. Reichenbach, *J. Antibiot.* **2000**, *53*, 579-558, and H. Reichenbach, G. Höfle, F. Sasse, H. Steinmetz (GBF), DE 196 38 870 A1, **1996**.



		R	R^1
1	Tubulysin A	iso-C_4H_9	OH
2	Tubulysin B	C_3H_7	OH
3	Tubulysin C	C_2H_5	OH
4	Tubulysin D	iso-C_4H_9	H
5	Tubulysin E	C_3H_7	H
6	Tubulysin F	C_2H_5	H

The problem of the invention is to make available new tubulysins, processes for the preparation thereof and preparations comprising tubulysins, especially as cytostatic agents.

An embodiment of the invention relates to a compound of the following general formula I (tubulysin):



R, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, S, T, U, V, W, X, Y and Z having the following meanings:

R = H, alkyl, aryl, OR^1, NR^1R^2 or NH-$(CH_2)_{2-4}$-

R^1 = H, alkyl or aryl
R^2 = H, alkyl or aryl
S = H, Hal, NO_2 or NHR^3
U = H, Hal, NO_2 or NHR^3
R^3 = H, HCO or C_{1-4}alkyl-CO
T = H or OR^4
R^4 = H, alkyl, aryl, COR^5, $P(O)(OR^6)_2$ or SO_3R^6
R^5 = alkyl, alkenyl, aryl or heteroaryl
R^6 = H, alkyl or a metal ion
V = H, OR^7, Hal or (with W) O
R^7 = H, alkyl or COR^8
R^8 = alkyl, alkenyl or aryl
W = H or alkyl or (with V) O
X = H, alkyl, alkenyl or CH_2OR^9
R^9 = H, alkyl, alkenyl, aryl or COR^{10}
R^{10} = alkyl, alkenyl, aryl or heteroaryl
Y = (for Z = CH_3 or COR^{11}) free electron pair or (for Z = CH_3) O
R^{11} = alkyl, CF_3 or aryl and/or
Z = (for Y = O or free electron pair) CH_3 or (for Y = free electron pair) COR^{11}.

Alkyl may be branched, unbranched or cyclic C_{1-20}alkyl, especially C_{1-7}alkyl, preferably C_{1-6}alkyl and more preferably C_{1-4}alkyl, especially methyl, ethyl, propyl, isopropyl, n-butyl,

isobutyl, sec-butyl, tert-butyl. Cycloalkyl has preferably from 3 to 8 carbon atoms in the ring.

Alkenyl groups may be branched, unbranched or cyclic C_{2-20}alkenyl, especially C_{2-7}alkenyl, preferably C_{2-6}alkenyl and more preferably C_{2-4}alkenyl, especially vinyl, allyl, propen-1-yl, propen-2-yl, but-1-en-1-yl, but-1-en-2-yl, but-1-en-3-yl, but-1-en-4-yl, but-2-en-1-yl, but-2-en-2-yl, 2-methyl-propen-1-yl, 2-methyl-propen-3-yl. Cycloalkenyl has preferably from 3 to 8 carbon atoms in the ring. The number of double bonds in the alkenyl groups may be from 1 to 3.

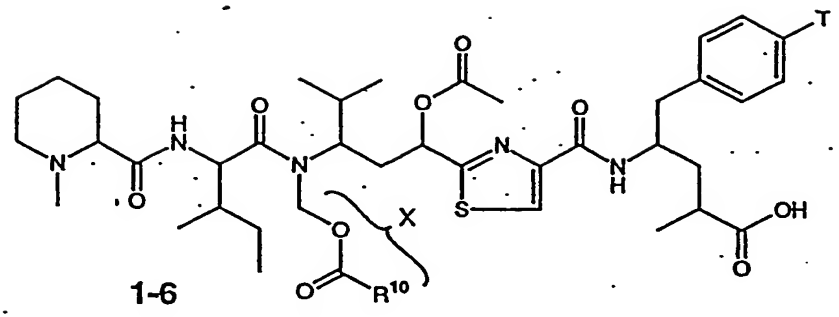
Aryl may be phenyl, naphthyl and biphenylyl.

Heteroaryl may be furyl, thienyl, imidazolyl, indolyl, pyridinyl, pyrrolyl, thiazolyl, oxazolyl and pyrimidinyl.

Alkyl, alkenyl, aryl and heteroaryl may be unsubstituted or substituted; accordingly they may carry, in any position, from 1 to 3 substituents from the group formed by C_{1-3}alkyl, C_{1-3}alkoxy, hydroxy, amino (NH_2) and nitro (NO_2).

A compound according to the invention may accordingly have:
R, R^1, R^4, R^5, R^8, R^9, R^{10} and/or R^{11} = unsubstituted or substituted phenyl, especially C_{1-4}alkyl-substituted phenyl
R^5 = C_{1-4}alkyl, C_{2-6}alkenyl or pyridyl
R^5 and/or X = C_{2-4}alkenyl
R^6 = an alkali metal ion, especially the Na ion, or an alkaline earth metal ion
R^8 and/or R^9 = C_{2-4}alkenyl and/or
R^{10} = C_{2-6}alkenyl, especially C_{2-4}alkenyl, or pyridyl.

A further embodiment of the invention (scheme 1) relates to a process for the preparation of a compound of the general formula I (type 7) wherein R = OR^1, R^1 = H, S = U = H, T = H or OH, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = H, Y = free electron pair and Z = CH_3, in which process a compound of the following general formula II (type 1, 2, 3, 4, 5 or 6):



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wherein X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl and which otherwise has the meanings indicated above is subjected to ester cleavage in an acidic medium and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the ester cleavage can be carried out in an organic solvent, especially dioxane, in the presence of an acid, especially hydrogen chloride, and/or at elevated temperature.

A further embodiment of the invention (scheme 1) relates to a process for the preparation of a compound of the general formula I (type 8) wherein R = OR^1, R^1 = H, S = U = H, T = H or OH, V = OR^7, R^7 = COR^8, R^8 = alkyl and especially C_{1-4}alkyl, especially methyl, W = H, X = H, Y = free electron pair and Z = CH_3, in which process a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) wherein X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl and which otherwise has the meanings indicated above is subjected to acetal cleavage and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the acetal cleavage can be carried out in an acidic medium, especially in the presence of hydrochloric acid, and/or at elevated temperature.

A further embodiment of the invention (scheme 1) relates to a process for the preparation of a compound of the general formula I (type 9) wherein R = OR^1, R^1 = H, S = U = H, T = H or OH, V = OR^7, R^7 = H, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, Y = free electron pair and Z = CH_3, in which process a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) wherein V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, and which otherwise has the meanings indicated above is

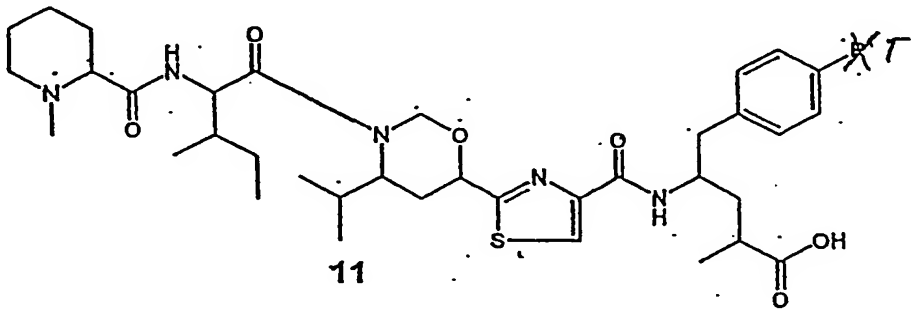
subjected to ester cleavage in a weakly alkaline medium and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the ester cleavage can be carried out in an organic medium, especially a hydrophilic organic solvent, preferably an alcohol, especially methanol, in the presence of a weak base, especially NH_3.

A further embodiment of the invention (scheme 1) relates to a process for the preparation of a compound of the general formula I (type 10) wherein R = OR^1, R^1 = H, S = U = H, T = H or OH, V = OR^7, R^7 = H, W = H, X = H, Y = free electron pair and Z = CH_3, in which process a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) wherein V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl and which otherwise has the meanings indicated above is subjected to double ester cleavage in a strongly alkaline medium and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the double ester cleavage can be carried out in an organic medium, especially in a hydrophilic organic solvent, preferably an alcohol, especially methanol, in the presence of a strong base, especially an alkali metal hydroxide, preferably sodium hydroxide.

A further embodiment of the invention (scheme 1) relates to a process for the preparation of a compound of the following general formula III (type 11):



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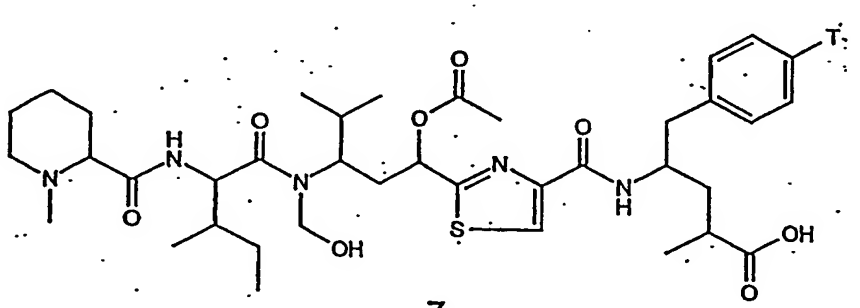
wherein R = OR^1, R^1 = H, S = U = H, T = H or OR^4, R^4 = H, V with X = CH_2O bridge, W = H, Y = free electron pair and Z = CH_3 in the general formula I, in which process a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) wherein X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, and which otherwise has the meanings indicated above is subjected to ring formation with double ester cleavage in an acidic medium and the compound of the general formula above having the indicated meanings is obtained.

In the process according to the invention, the ring formation can be carried out in an aqueous medium, in the presence of an inorganic acid, preferably hydrochloric acid, and with heating.

A further embodiment (scheme 2) relates to a process for the preparation of a compound of the general formula I (type 12) wherein R = OR^1, R^1 = H, S = U = H, T = H or OR^4, R^4 = COR^5, R^5 = alkyl and especially C_{1-6}alkyl, alkenyl and especially C_{2-6}alkenyl, aryl or heteroaryl, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = R^5, Y = free electron pair and Z = CH_3, in which process a compound of the following general formula IV (type 7):



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wherein X = CH_2OR^9, R^9 = H and which otherwise has the meanings indicated above is subjected to acylation and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the acylation can be carried out using an acyl halide, especially an acyl chloride, and/or in the presence of a weak base, especially a weak organic base, preferably a tertiary amine, especially triethylamine.

A further embodiment of the invention (scheme 2) relates to a process for the preparation of a compound of the general formula I (type 13) wherein R = OR^1, R^1 = H, S = U = H, T = H or OR^4, R^4 = H, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl and especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair and Z = CH_3, in which process hydrolysis is carried out in an alkaline medium on a product according to the invention wherein T = OR^4, R^4 = COR^5 and R^5 = alkyl and especially C_{1-6}alkyl, alkenyl and especially C_{2-6}alkenyl, aryl or heteroaryl and which otherwise has the meanings indicated above, and a compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the hydrolysis can be carried out using ammonia.

A further embodiment of the invention (scheme 3) relates to a process for the preparation of a compound of the general formula I (type 14) wherein R = DR^1, R^1 = H, S = U = H, T = H or OH, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = alkyl and especially C_{1-4}alkyl, alkenyl and especially C_{2-6}alkenyl or aryl, Y = free electron pair and Z = CH_3, in which process a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) is subjected to ester cleavage and is alkylated and a compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the reaction can be carried out using an alkylating agent of formula R^9OH wherein R^9 = alkyl and especially C_{1-4}alkyl, alkenyl or aryl.

In the process according to the invention, the reaction can be carried out in the presence of p-CH_3-$C_6H_4SO_2OH$ in tetrahydrofuran (THF) at elevated temperature.

A further embodiment of the invention (scheme 4) relates to a process for the preparation of a compound of the general formula I (type 15) wherein R = OR^1, R^1 = H, S = U = H, T = H or OR^4, R^4 = H, V = OR^7, R^7 = H or COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_3, Y = free electron pair and Z = CH_3, in which process a compound of the general formula IV (type 7) wherein X = CH_2OR^9, R^9 = H and which otherwise has the meanings indicated above is subjected to reduction and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the reduction can be carried out using $NaCNBH_3$ and trifluoroacetic acid in methanol (MeOH).

A further embodiment of the invention (scheme 4) relates to a process for the preparation of a compound of the general formula I (type 15) wherein R = OR^1, R^1 = H, S = U = H, T = H or OR^4, R^4 = H, V = OR^7, R^7 = H or COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_3, Y = free electron pair and Z = CH_3, in which process a compound of the general formula III (type 11) is subjected to ring opening with reduction or to reduction with ring opening and a compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the reaction can be carried out in the presence of $NaCNBH_3$ and Me_3SiCl in acetonitrile (CH_3CN).

A further embodiment of the invention (scheme 5) relates to a process for the preparation of a compound of the general formula I (type 16) wherein R = OR^1, R^1 = H, S = U = H, T = H or OH, V = OR^7, R^7 = COR^8, R^8 = alkyl and especially C_{1-4}alkyl, alkenyl or aryl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl or alkenyl, Y = free electron pair and Z = CH_3, in which process a compound of the general formula I according to the invention (type 9) wherein V = OR^7 and R^7 = H and which otherwise has the meanings indicated above is subjected to acylation and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the acylation can be carried out using an acyl halide of formula R^8COCl wherein R^8 = alkyl and especially C_{1-4}alkyl, alkenyl or aryl, especially an acyl chloride, and/or in the presence of a base, especially an organic base, preferably a trialkylamine, especially triethylamine.

A further embodiment of the invention (scheme 5) relates to a process for the preparation of a compound of the general formula I (type 17) wherein R = OR^1, R^1 = H, S = U = H, T = H or OR^4, R^4 = H, V = H or F, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl or alkenyl, Y = free electron pair and Z = CH_3, in which process a compound of the general formula I according to the invention (type 9) wherein V = OR^7 and R^7 = H and which otherwise has the meanings indicated above is subjected to catalytic hydrogenation

or fluorination and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, for V = H, the hydrogenation can be carried out using palladium-on-carbon in the presence of acetic acid or, for V = F, the fluorination can be carried out using DAST in tetrahydrofuran.

A further embodiment of the invention (scheme 5) relates to a process for the preparation of a compound of the general formula I (type 18) wherein R = OR^1, R^1 = H, S = U = H, T = H or OR^4, R^4 = H, V with W = O, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl or alkenyl, Y = free electron pair and Z = CH_3, in which process a compound of the general formula I according to the invention (type 9) wherein V = OR^7 and R^7 = H and which otherwise has the meanings indicated above is subjected to oxidation with formation of a ketone and a compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the oxidation can be carried out in the presence of TPAP and NMO in dichloromethane.

A further embodiment of the invention (scheme 5) relates to a process for the preparation of a compound of the general formula I (type 19) wherein R = OR^1, R^1 = H, S = U = H, T = H or OH, V = OR^7, R^7 = H, W = alkyl and especially C_{1-4}alkyl, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl or alkenyl, Y = free electron pair and Z = CH_3, in which process a product of the above process according to the invention (type 18) is reacted with a Grignard compound to form the compound of the general formula I having the indicated meanings.

In the process according to the invention, the reaction can be carried out using an organomagnesium compound of formula WMgHal wherein W = alkyl and especially C_{1-4}alkyl.

A further embodiment of the invention (scheme 5) relates to a process for the preparation of a compound of the general formula I (type 19) wherein R = OR^1, R^1 = H, S = U = H, T = H or OH, V = OR^7, R^7 = H, W = alkyl and especially C_{1-4}alkyl, X = CH_2OR^9, R^9 = COR^{10}, R^{10}

= alkyl and especially C_{1-6}alkyl or alkenyl, Y = free electron pair and Z = CH_3, in which process
(i) in a first step a process according to the invention is carried out and a compound according to the invention (type 18) is obtained and then
(ii) in a second step the resulting compound according to the invention (type 18) is reacted in a further process according to the invention to form a compound of the general formula I having the indicated meanings and that compound is obtained.

A further embodiment of the invention (scheme 6) relates to a process for the preparation of a compound of the general formula I (type 20) wherein R = OR^1, R^1 = alkyl and especially C_{1-4}alkyl or alkenyl, S = U = H, T = H or OR^4, R^4 = H, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl and especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair and Z = CH_3, in which process a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) or a product of a process according to the invention (type 13) is subjected to alkylation or alkenylation and a compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the alkylation or alkenylation can be carried out in the presence of EDC, R^1OH wherein R^1 = alkyl and especially C_{1-4}alkyl or alkenyl, and DMAP in methylene chloride.

A further embodiment of the invention (scheme 6) relates to a process for the preparation of a compound of the general formula I (type 21) wherein R = NHR^1, NH-NR^1R^2, $NHOR^1$ or $NH(CH_2)_{2-4}NR^1R^2$, R^1 and R^2 each independently of the other = H, alkyl and especially C_{1-6}alkyl or aryl, S = U = H, T = H or OR^4, R^4 = H, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl and especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair and Z = CH_3, in which process a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) or a product of a process according to the invention (type 13) is subjected to amination using a compound of formula RH, R having the indicated meanings, and a compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the reaction can be carried out
(i) in the presence of EDC in methylene chloride or

(ii) in the presence of isobutyl chloroformate and triethylamine in THF.

A further embodiment of the invention (scheme 6) relates to a process for the preparation of a compound of the general formula I (type 22) wherein R = alkyl and especially C_{1-4}alkyl or alkenyl, S = U = H, T = H or OR^4, R^4 = H, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl and especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair and Z = CH_3, in which process a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) or a product of a process according to the invention (type 13) is reacted with an organolithium compound of formula RLi having the indicated meaning for R to form the compound of the general formula I having the indicated meanings.

A further embodiment of the invention (scheme 6) relates to a process for the preparation of a compound of the general formula I (type 23) wherein R = amino radical of 1-(2-amino-C_{2-4}alkyl)-pyrrole-2,5-dione, S = U = H, T = H or OR^4, R^4 = H, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl and especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair and Z = CH_3, in which process a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) or a product of a process according to the invention (type 13) is subjected to amination using 1-(2-amino-C_{2-4}alkyl)-pyrrole-2,5-dione and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the amination can be carried out in the presence of EDC in methylene chloride.

A further embodiment of the invention (scheme 7) relates to a process for the preparation of a compound of the general formula I (type 24) wherein R = OR^1, R^1 = H, S = U = H, T = OR^4, R^4 = $P(O)(OR^6)_2$ wherein R^6 = H or alkyl, especially C_{1-4}alkyl, or R^4 = SO_3R^6 wherein R^6 = H, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair and Z = CH_3, in which process
(i) a compound of the general formula II (type 1, 2 or 3) or
(ii) a product of a process according to the invention (type 13)
is reacted with

(a) a compound of formula $P(O)(OR^6)_2OH$ wherein R^6 = H or alkyl and especially C_{1-4}alkyl
or
(b) SO_3
and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the variant (a) can be carried out in the presence of I_2 and pyridine in methylene chloride.

In the process according to the invention, the variant (b) can be carried out using pyridine-SO_3.

A further embodiment of the invention (scheme 7) relates to a process for the preparation of a compound of the general formula I (type 25) wherein R = OR^1, R^1 = H, S = U = H, T = OR^4, R^4 = COR^5, R^5 = alkyl and especially C_{1-4}alkyl, alkenyl or $N(R^{12})_2$, R^{12} = alkyl, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl, especially C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, in which process
(i) a compound of the general formula II (type 1, 2 or 3) or
(ii) a product of a process according to the invention (type 13)
is subjected to acylation and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the acylation can be carried out using an acyl halide of formula R^5COCl wherein R^5 = alkyl and especially C_{1-4}alkyl, alkenyl or $N(R^{12})_2$ and R^{12} = alkyl, especially using an acyl chloride, in the presence of an organic base, especially a trialkylamine, preferably triethylamine, in an organic solvent, especially THF.

A further embodiment of the invention (scheme 7) relates to a process for the preparation of a compound of the general formula I (type 26) wherein R = OR^1, R^1 = alkyl and especially C_{1-4}alkyl or alkenyl, S = U = H, T = OR^4, R^4 = alkyl and especially C_{1-4}alkyl or alkenyl, V = OR^7, R^7 = COR^8, R^8 = alkyl and especially C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl, especially C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair and Z = CH_3, in which process
(i) a compound of the general formula II (type 1, 2 or 3) or
(ii) a product of a process according to the invention (type 13)

is subjected to alkylation and the compound of the general formula according to claim 1 having the indicated meanings is obtained.

In the process according to the invention, the alkylation can be carried out using an alkyl iodide of formula R^4I wherein R^4 = alkyl and especially C_{1-4}alkyl or alkenyl in the presence of a weak base, especially Ag_2O, in an organic solvent, especially methylene chloride.

In the process according to the invention, methylation can be carried out using diazomethane in an organic solvent, especially methanol.

A further embodiment of the invention (scheme 7) relates to a process for the preparation of a compound of the general formula I (type 27) wherein R = OR^1, R^1 = H, S = U = H, T = OR^4, R^4 = alkyl and especially C_{1-4}alkyl or alkenyl, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl, especially C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair and Z = CH_3, in which process a product of a process according to the invention (type 26) is subjected to partial dealkylation or dealkenylation enzymatically and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, an esterase, especially pig liver esterase, can be used as the enzyme.

A further embodiment of the invention (scheme 7) relates to a process for the preparation of a compound of the general formula I (type 27) R = OR^1, R^1 = H, S = U = H, T = OR^4, R^4 = alkyl and especially C_{1-4}alkyl or alkenyl, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, in which process
(a) in a first step
(i) a compound of the general formula II (type 1, 2 or 3) or
(ii) a product of a process according to the invention (type 13)
is subjected to a process according to the invention and a compound according to the invention (type 26) is obtained and
(b) in a second step the resulting compound according to the invention (type 26) is reacted in a further process according to the invention to form a compound of the general formula I having the indicated meanings and that compound is obtained.

A further embodiment of the invention (scheme 8) relates to a process for the preparation of a compound of the general formula I (type 28 and, as the case may be, 29) wherein R = OR^1, R^1 = H, S = H or Hal, T = OR^4, R^4 = H, U = Hal, V = OR^7, R^7 = COR^8, R^8 = alkyl and especially C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, in which process
(i) a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) or
(ii) a product of a process according to the invention (type 13)
is subjected to halogenation or dihalogenation in the position ortho to the T substituent and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the halogenation can be carried out in the presence of C_5Cl_5NF-triflate, SO_2Cl_2, NBS and ICl.

A further embodiment of the invention (scheme 8) relates to a process for the preparation of a compound of the general formula I (type 30) wherein R = OR^1, R^1 = H, S = H, T = OR^4, R^4 = H, U = NO_2, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl, especially C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair and Z = CH_3, in which process
(i) a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) or
(ii) a product of a process according to the invention (type 13)
is subjected to nitration in the position ortho to the T substituent and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the nitration can be carried out using an alkali metal nitrite, especially sodium nitrite, and acetic acid in the presence of an organic solvent, especially ethanol.

A further embodiment of the invention (scheme 8) relates to a process for the preparation of a compound of the general formula I (type 31) wherein R = OR^1, R^1 = H, S = H, T = OR^4, R^4 = H, U = NH_2, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair and Z = CH_3, in which process a product of a process according to the invention (type 30) is subjected to catalytic reduction and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the reduction can be carried out using elemental hydrogen in the presence of palladium on activated carbon, especially in an organic solvent, preferably ethanol.

A further embodiment of the invention (scheme 8) relates to a process for the preparation of a compound of the general formula I (type 31) wherein R = OR^1, R^1 = H, S = H, T = OR^4, R^4 = H, U = NH_2, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair and Z = CH_3, in which process
(a) in a first step
(i) a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) or
(ii) a product of a process according to the invention (type 13)
is subjected to a further process according to the invention and a compound according to the invention (type 30) is obtained and
(b) in a second step the resulting product (type 30) is subjected to a further process according to the invention and the compound of the general formula I having the indicated meanings is obtained.

A further embodiment of the invention (scheme 8) relates to a process for the preparation of a compound of the general formula I (type 32) wherein R = OR^1, R^1 = H, S = H, T = OR^4, R^4 = H, U = NHR^3, R^3 = alkyl-CO and especially C_{1-4}alkyl-CO, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair and Z = CH_3, in which process a product of a process according to the invention (type 31) is subjected to alkylation and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the alkylation can be carried out using an acid anhydride of formula $(R^3)_2O$ wherein R^3 = CO-C_{1-4}alkyl.

A further embodiment of the invention (scheme 8) relates to a process for the preparation of a compound of the general formula I (type 32) wherein R = OR^1, R^1 = H, S = H, T = OR^4, R^4 = H, U = NHR^3, R^3 = alkyl-CO and especially C_{1-4}alkyl-CO, V = OR^7, R^7 = COR^8, R^8 =

alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl, preferably C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, in which process
(a) in an optional first step
(i) a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) or
(ii) a product of a process according to the invention (type 13)
is subjected to a further process according to the invention,
(b) in a second step the resulting product (type 30) is subjected to a further process according to the invention and
(c) in a third step the resulting compound according to the invention (type 31) is subjected to a further process according to the invention and
the compound of the general formula I having the indicated meanings is obtained.

A further embodiment of the invention (scheme 9) relates to a process for the preparation of a compound of the general formula I (type 33) wherein R = OR^1, R^1 = H, S = U = H, T = OR^4, R^4 = H, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, Y = O and Z = CH_3, in which process
(i) a compound of the general formula II (type 1, 2, 3, 4, 5 or 6) or
(ii) a product of a process according to the invention (type 13)
is subjected to a reaction for formation of an N-oxide and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the N-oxide formation can be carried out using m-CPBA in an organic solvent, especially methylene chloride.

A further embodiment of the invention (scheme 9) relates to a process for the preparation of a compound of the general formula I (type 34) wherein R = OR^1, R^1 = H, S = U = H, T = OR^4, R^4 = H, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair, Z = COR^{11} and R^{11} = alkyl, preferably C_{1-4}alkyl, especially methyl, in which process the product of a process according to the invention (type 33) is reacted with an acylating agent and the compound of the general formula I having the indicated meanings is obtained.

In the process according to the invention, the acylation can be carried out using an acid anhydride, especially acetic anhydride, preferably at elevated temperature.

A further embodiment of the invention (scheme 9) relates to a process for the preparation of a compound of the general formula I (type 34) wherein R = OR^1, R^1 = H, S = U = H, T = OR^4, R^4 = H, V = OR^7, R^7 = COR^8, R^8 = alkyl, preferably C_{1-4}alkyl, especially methyl, W = H, X = CH_2OR^9, R^9 = COR^{10}, R^{10} = alkyl and especially C_{1-6}alkyl, alkenyl, especially C_{2-6}alkenyl, aryl or heteroaryl, Y = free electron pair, Z = COR^{11} and R^{11} = alkyl, preferably C_{1-4}alkyl, especially methyl, in which process
(a) in a first step
(i) a compound of the general formula II (type 1, 2, 3, 4, 5, or 6) or
(ii) a product of a process according to the invention (type 13) is subjected to a process according to the invention and
(b) in a second step the resulting product (type 33) is subjected to a further process according to the invention and
the compound of the general formula according to claim 1 having the indicated meanings is obtained.

A further embodiment of the invention relates to a therapeutic preparation, especially a cytostatic agent, comprising one or more compounds according to the invention as active ingredient in addition to one or more optional customary carriers and/or one or more optional customary diluents.

Finally, an embodiment of the invention relates to a therapeutic preparation, especially a cytostatic agent, comprising one or more products of a process according to the invention as active ingredient in addition to one or more optional customary carriers and/or one or more optional customary diluents.

The invention is described hereinbelow in greater detail by means of Examples.

*Tubulysin derivative **7a**: R^1 = OH (scheme 1)*

9.9 mg (11.7 µmol) of tubulysin A (**1**) were dissolved in 200 µl of dioxane and 1 ml of 0.1M hydrochloric acid solution was added. The reaction mixture was stirred at 50°C for 8 hours. The mixture was then lyophilised and the residue subjected to preparative HPLC (CH_3CN/H_2O 35/65 with 50mM NH_4Ac, pH = 6.5), whereupon 5.3 mg (59 %) of **7a** were obtained.

R_f 0.55; $[\alpha]^{22}_D$ -7.0 (c 0.89 MeOH); **UV** (MeOH): λ_{max} nm (lgε) 226 (4.13), 250 (3.91); **IR** (KBr): ν_{max} 3386, 2963, 2934, 1655, 1546, 1232 cm^{-1}; **^{1}H NMR** (DMSO-d_6 600 MHz): as tubulysin A (**1**) except Tuv δ 8.06 (1H, s, H-3), 6.18 (1H, d, J = 11.9 Hz, H-11b), 5.37 (1H, d, J = 11.8 Hz, H-11a), 4.63 (1H, br, H-5), 4.10 (1H, br, H-7), 2.20 (1H, m, H-6b), 1.99 (1H, m, H-8), 1.98 (1H, m, H-6a), 1.91 (1H, m, H-2'b), 1.48 (1H, m, H-3'b), 1.44 (1H, m, H-3'a), 1.42 (1H, m, H-2'a), 0.92 (3H, d, J = 6.4 Hz, H-9), 0.80 (3H, t, J = 7.3 Hz, H-4'), 0.73 (3H, d, J = 6.2 Hz, H-10); **^{13}C NMR** (DMSO-d_6 150 MHz): as tubulysin A (**1**) except Tuv δ 178.0 (s, C-4), 174.4 (s, C-1'), 160.0 (s, C-1), 149.6 (s, C-2), 123.0 (d, C-3), 68.0 (t, C-11), 67.5 (d, C-5), 55.0 (d, C-7), 37.4 (t, C-2'), 35.7 (t, C-6), 30.6 (d, C-8), 20.1 (q, C-9), 19.5 (q, C-10), 17.7 (t, C-3'), 13.3 (q, C-4'); **DCI MS**: m/z [M+H$^+$] 760 (4); **HRMS (DCI)**: $C_{38}H_{58}N_5O_9S$: 760.3917 $[M+H]^+$ (calc.: 760.3955).

*Tubulysin derivative **8a**: R^1 = OH (scheme 1)*

500 µl of 0.1M hydrochloric acid were added to 20.0 mg (23.7 µmol) of tubulysin A (**1**). The reaction mixture was stirred for 5 minutes at 100°C, was then cooled and was neutralised (pH = 7) using saturated $NaHCO_3$ solution. Extraction was then carried out three times using ethyl acetate, and the combined organic phases were concentrated. The crude product was purified by means of preparative HPLC (CH_3CN/H_2O 35/65 with 50mM NH_4Ac, pH = 6.5), whereupon 6.4 mg (37 %) of **8a**, 2.7 mg (15 %) of **7a** and 5.1 mg (31 %) of **10a** were obtained.

8a:

R_f 0.55; $[\alpha]^{22}_D$ -10.2 (c 1.0 MeOH); **UV** (MeOH): λ_{max} nm (lgε) 225 (4.10), 250 (3.94); **IR** (KBr): ν_{max} 3389, 3251, 2962, 2934, 1658, 1547, 1228 cm^{-1}; **^{1}H NMR** (DMSO-d_6 600 MHz): as tubulysin A (**1**) except Tuv δ 8.17 (1H, s, H-3), 7.92 (1H, br, NH-7), 5.76 (1H, dd, J = 10.5, 3.0 Hz, H-5), 3.86 (1H, m, H-7), 2.13 (1H, m, H-6b), 2.09 (3H, s, H-50Ac), 1.95 (1H, m, H-6a), 1.73 (1H, m, H-8), 0.84 (3H, d, J = 6.4 Hz, H-10), 0.83 (3H, d, J = 6.0 Hz, H-9), Ile δ 7.54 (1H, d, J = 9.3 Hz, NH-2), 4.18 (1H, dd, J = 9.1 Hz, H-2), 1.75 (1H, m, H-3), 1.48 (1H, m, H-4b), 1.07 (1H, m, H-4a), 0.85 (3H, m, H-6), 0.81 (3H, m, H-5); **^{13}C NMR** (DMSO-d_6 150 MHz): as tubulysin A (**1**) except Tuv δ 169.6 (s, C-50Ac), 169.6 (s, C-4), 159.8 (s, C-1), 149.8 (s, C-2), 124.0 (d, C-3), 69.5 (d, C-5), 49.5 (d, C-7), 36.4 (t, C-6), 31.7 (d, C-8), 20.6 (q, C-50Ac), 18.9 (q, C-9), 18.0 (q, C-10), Ile δ 171.1 (s, C-1), 56.7 (d, C-2), 36.2 (d, C-3), 24.3 (t, C-4), 15.6 (q, C-6), 10.6 (q, C-5); **DCI MS**: m/z [M+H$^+$] 730 (100), 672 (15); **HRMS (DCI)**: $C_{37}H_{56}N_5O_8S$: 730.3839 $[M+H]^+$ (calc.: 730.3850).

*Tubulysin derivative **9a**: R = iso-C_4H_9, R^1 = OH (scheme 1)*

9.6 mg (11.4 µmol) of tubulysin A (**1**) were dissolved in 1 ml of methanol and, at intervals of three hours, 10 µl (133.6 µmol) of 25 % ammonia were added on each occasion and stirring was carried out at room temperature. The reaction mixture was then adjusted to pH 5 using 18 % hydrochloric acid and extracted three times with ethyl acetate. The combined organic phases were concentrated and purified by means of PLC (CH_2Cl_2/MeOH 85/15). 2.5 mg (27 %) of **9a**, 2.3 mg (29 %) of **10a** and 1.7 mg (18 %) of **1** were isolated.

9a:

ESI MS (1 eV): 802 $[M+H]^+$; **^{1}H NMR** (DMSO-d_6, 600 MHz): δ = 4.63 (br, 1H, H-5), 4.10 (br, 1H, H-7), 2.20 (m, 1H, H-6b), 1.99 (m, 1H, H-8), 1.98 (m, 1H, H-6a).

*Tubulysin derivative **10a**: R^1 = OH (scheme 1)*

5.4 mg (6.8 µmol) of tubulysin A (**1**) were dissolved in 300 µl of methanol, 67.0 µl of 1M sodium hydroxide solution (67.6 µmol) were added and stirring was carried out for 15 minutes at room temperature. The reaction mixture was then diluted with water and adjusted to pH 7 using 1M hydrochloric acid solution. After extracting three times with ethyl

acetate, the combined organic phases were concentrated. The residue was purified by means of preparative HPLC (CH_3CN/H_2O 35/65 with 50mM NH_4Ac, pH = 6.5), whereupon 2.5 mg (57 %) of **10a** were obtained.

R_f 0.40; $[\alpha]^{22}_D$ -3.0 (c 0.66 MeOH); **UV** (MeOH): λ_{max} nm (lg ε) 225 (4.12), 250 (3.92); **IR** (KBr): ν_{max} 3376 cm^{-1}, 3285, 2960, 2929, 1656, 1547; **^{1}H NMR** (DMSO-d_6 600 MHz): as tubulysin A (**1**) except Tuv δ 8.06 (1H, s, H-3), 7.67 (1H, br, NH-7), 4.65 (1H, ddbr, J = 8.7 Hz, H-5), 3.97 (1H, m, H-7), 1.98 (1H, m, H-6b), 1.79 (1H, m, H-6a), 1.74 (1H, m, H-8), 0.85 (3H, d, J = 6.7 Hz, H-9), 0.84 (3H, d, J = 6.2 Hz, H-10), Ile δ 7.75 (1H, br, NH-2), 4.15 (1H, dd, J = 8.6 Hz, H-2), 1.81 (1H, m, H-3), 1.55 (1H, m, H-4b), 1.11 (1H, m, H-4a), 0.86 (3H, d, J = 6.4 Hz, H-6), 0.80 (3H, t, J = 7.2 Hz, H-5); **^{13}C NMR** (DMSO-d_6 150 MHz): as tubulysin A (**1**) except Tuv δ 177.9 (s, C-4), 160.1 (s, C-1), 149.7 (s, C-2), 122.8 (d, C-3), 67.9 (d, C-5), 50.3 (d, C-7), 40.5 (t, C-6), 31.8 (d, C-8), 19.0 (q, C-9), 18.1 (q, C-10), Ile δ 171.4 (s, C-1), 57.1 (d, C-2), 36.2 (d, C-3), 24.6 (t, C-4), 15.6 (q, C-6), 10.4 (q, C-5); **DCI MS**: m/z $[M+H^+]$ 688 (100), 256 (12), 223 (6), 98 (4); **HRMS (DCI)**: $C_{35}H_{54}N_5O_7S$: 688.3799 $[M+H]^+$ (calc.: 688.3744).

*Methyl ester of tubulysin derivative **10a**:*

2.5 mg (3.6 µmol) of 10a were dissolved in 200 µl of methanol; ethereal diazomethane solution was added and stirring was carried out for 10 minutes at room temperature. Purification was carried out directly by means of PLC (CH_2Cl_2/MeOH 85/15) and yielded 2.8 mg (62 %) of the methyl ester of **10a**.

R_f (CH_2Cl_2/MeOH 85/15): 0.32; **IR** (KBr): $\tilde{\nu}$= 3380 cm^{-1} (m), 2960 (s), 2931 (s), 2872 (w), 1743 (w), 1659 (vs), 1516 (m), 1371 (w), 1229 (s), 1092 (w); **UV** (MeOH): λ_{max} (lg ε) = 204 nm (4.53), 226 (4.27), 244 (sh, 4.01), 276 (sh, 3.38); **DCI MS** (120 eV, NH_3): 702 $[M+H]^+$; **^{1}H NMR** (CD_3OD, 300 MHz): δ = 1.18 (d, 3 H, Tut10-H), 3.64 (s, 3 H, Tut11-H).

*Cyclo-tubulysin A (**11a**): R^1 = OH (scheme 1)*

1 ml of 0.5M hydrochloric acid solution was added to 9.6 mg (11.3 µmol) of tubulysin A (**1**) (distributed as a thin film on the glass walls of the reaction vessel) and stirring was carried out for 30 minutes at 100°C. The reaction mixture was then lyophilised and the residue was purified by means of PLC (CH_2Cl_2/MeOH 90/10), whereupon 3.9 mg (50 %) of **11a** and 1.6 mg (21 %) of **10a** were obtained.

11a:

ESI MS (1 eV): 699 $[M+H]^+$; **^{1}H NMR** (DMSO-d_6, 300 MHz): δ = 8.15 (s, 1 H, Tuvt3-H), 5.32 (d, 1 H, Tuv5-H), 1.7 (m, 1 H, Tuv6a-H), 2.3 (m, 1 H, Tuv6b-H), 4.32 (m, 1 H, Tuv7-H), 1.8 (m, 1 H, Tuv8-H), 0.75 (d, 3 H, Tuv9-H_3), 0.95 (d, 3 H, Tuv10-H_3), 4.86 (d, 1 H, Tuv11a-H), 5.65 (d, 1 H, Tuv11b-H).

*Tubulysin derivatives **12 and 13***

A tubulysin derivative 12 can be prepared from a tubulysin derivative 7a by reacting 7a with acetyl chloride in triethylamine.

By starting from a tubulysin derivative of type 7 wherein T = OR^4, R^4 = COR^5 and R^5 = methyl or ethyl, the resulting tubulysin derivative of type 12 can be hydrolysed, using ammonia, to form a tubulysin derivative of type 13.

*Tubulysin A methyl ether (**14a**): R^1 = OH (scheme 3)*

500 µl of absolute ethanol and 1 mg (5.3 µmol) of *p*-toluenesulfonic acid were added to a solution of 10.0 mg (11.9 µmol) of tubulysin A (**1**) in 500 µl of absolute THF. The reaction mixture was stirred for 20 minutes at 80°C. The solvent was then removed and the crude product was purified by means of PLC (CH_2Cl_2/MeOH 90/10). 3.1 mg (33 %) of **14a** were obtained.

ESI MS (1 eV): 788 $[M+H]^+$

*Tubulysin derivative **15***

A tubulysin derivative 15 can be prepared by reducing a tubulysin derivative 7a using $NaCNBH_3$ and TFA in methanol.

*Tubulysin derivative **16***

A tubulysin derivative 16 can be prepared by acetylating a tubulysin derivative 9a using acetyl chloride in triethylamine.

*Tubulysin derivative **17***

A tubulysin derivative 17 can be prepared by catalytically hydrogenating a tubulysin derivative 9a in the presence of CH_3COOH/DAST using a Pd/C catalyst and elemental hydrogen.

*Tubulysin derivatives **18 and 19***

A tubulysin derivative 18 can be prepared by oxidising a tubulysin derivative 9a in the presence of TPAP and NMO.

The tubulysin derivative 18 obtained can be reacted with ethylmagnesium bromide to form a tubulysin derivative 19.

*Tubulysin A methyl ester (**20a**): $R^2 = CH_3$ (scheme 6)*

19.0 mg (22.5 µmol) of tubulysin A (**1**) were dissolved in 300 µl of methanol, and ethereal diazomethane solution was added at room temperature on two occasions spaced 15 minutes apart. The reaction mixture was concentrated and purification of the crude

product was carried out by means of PLC (CH_2Cl_2/MeOH 90/10), whereupon 11.7 mg (61 %) of **20a** were obtained.

IR (KBr): $\tilde{\nu}$= 3383 cm^{-1} (m), 2962 (s), 2875 (w), 1739 (vs), 1666 (vs), 1516 (s), 1227 (vs), 1091 (w); **UV** (MeOH): λ_{max} (lg ε) = 203 nm (4.61), 225 (4.34), 243 (sh, 4.11), 276 (sh, 3.41); **DCI MS** (120 eV, isobutane): 858 $[M+H^+]$; **^{1}H NMR** (DMSO-d_6, 300 MHz): δ = 2.43 (m, 1 H, Tut2-H), 1.06 (d, 3 H, Tut10-H), 3.53 (s, 3 H, Tut11-H); **^{13}C NMR** (DMSO-d_6, 75 MHz): δ = 175.8 (TutC1), 35.8 (TutC2), 17.6 (TutC10), 51.3 (TutC11).

*Tubulysin A ethyl ester (**20b**): R^1 = OH, R^2 = C_2H_5 (scheme 6)*

To a solution of 5.2 mg (6.2 µmol) of tubulysin A (**1**) in 300 µl of dichloromethane there were added 13.5 µl (9.3 µmol) of ethanol, 1.8 mg (9.3 µmol) of EDC and 57 µl (9.3 µmol) of DMAP solution (5 mg/250 µl of CH_2Cl_2). The reaction mixture was stirred overnight at room temperature. Purification of the crude product was then carried out by means of PLC (CH_2Cl_2/MeOH 90/10), whereupon 1.7 mg (32 %) of **20b**, 0.7 mg (13 %) of **25a** and 1.0 mg (18 %) of **20b** wherein R^1 = $OCOCH_3$ were obtained.

20b:

DCI MS (120 eV, NH_3): 872 $[M+H]^+$; **HRMS (DCI):** $C_{45}H_{69}N_5O_{10}S$: $[M+H]^+$ calc.: 872.4843 (found: 872.4917): **^{1}H NMR** (CD_3OD, 300 MHz): δ = 2.58 (m, 1 H, Tut2-H, 1.19 (d, 3 H, Tut10-H), 4.11 (q, 2 H, Tut11-H), 1.23 (t, 3 H, Tut12-H).

20b wherein R^1 = $OCOCH_3$

R_f (CH_2Cl_2/MeOH 90/10): 0.51; **IR** (KBr): $\tilde{\nu}$= 3392 cm^{-1} (m), 2962 (s), 2920 (s), 2874 (w), 1736 (s), 1667 (vs), 1507 (m), 1370 (w), 1218 (s), 1195 (s); **UV** (MeOH): λ_{max} (lg ε) = 203 nm (4.60), 218 (4.30), 227 (sh, 4.23), 248 (sh, 3.98); **DCI MS** (120 eV, NH_3): 914 $[M+H^+]$; **HRMS (DCI):** $C_{47}H_{71}N_5O_{11}S$: $[M+H]^+$ calc.: 814.4949 found: 914.5044; **^{1}H NMR** (CD_3OD, 300 MHz): δ = 7.29 (d, 2 H, Tut7-H), 7.02 (d, 2 H, Tut8-H), 1.20 (d, 3 H, Tut10-H), 4.11 (q, 2 H, Tut11-H), 1.23 (t, 3 H, Tut12-H), 2.28 (s, 3-H, Tut13-H).

*Tubulysin A propyl ester (**20c**): R^1 = OH, R^2 = C_3H_7 (scheme 6)*

To a solution of 11.3 mg (13.4 µmol) of tubulysin A (**1**) in 450 µl of dichloromethane/diethyl ether (1/2) there were added 6.5 µl (67.0 µmol) of propyl iodide and 6.2 mg (26.8 µmol) of silver(I) oxide. The reaction mixture was stirred overnight at room temperature and then filtered over Celite, and the residue was washed with dichloromethane. The combined organic phases were concentrated and purified by means of PLC (CH_2Cl_2/MeOH 90/10), whereupon 7.6 mg (64 %) of **20c** were obtained.

IR (KBr): $\tilde{\nu}$= 3387 cm^{-1} (m), 2964 (s), 2938 (m), 2876 (w), 1737 (s), 1667 (vs), 1516 (s), 1416 (m), 1370 (w), 1225 (s), 1094 (w); **UV** (MeOH): λ_{max} (lg ε) = 204 nm (4.64), 224 (4.39), 246 (sh, 4.12), 276 (3.60); **DCI MS** (120 eV, NH_3): 886 [M+H^+]; **^{1}H NMR** (CD_3OD, 300 MHz): δ = 2.63 (m, 1 H, Tut2-H), 1.68 (m, 1 H, Tut3a-H), 2.03 (m, 1 H, Tut3b-H), 4.32 (m, 2 H, Tut4-H), 2.83 (m, 2 H, Tut5-H), 6.72 (d, 1 H, Tut7-H), 7.08 (d, 1 H, Tut8-H), 1.20 (d, 3 H, Tut10-H), 4.02 (t, 2 H, Tut11-H), 1.64 (m, 2 H, Tut12-H), 0.95 (t, 3 H, Tut13-H); **^{13}C NMR** (CD_3OD, 75 MHz): δ = 177.9 (TutC1), 38.1 (TutC2), 38.9 (TutC3), 50.7 (TutC4), 41.5 (TutC5), 130.0 (TutC6), 116.2 (TutC7), 131.4 (TutC8), 157.1 (TutC9), 18.4 (TutC10), 67.2 (TutC11), 23.0 (TutC12), 10.7 (TutC13).

*Tubulysin A propylamide (**21a**): R^1 = OH, R^2 = C_3H_7 (scheme 6)*

To a solution of 4.9 mg (5.8 µmol) of tubulysin A (**1**) in 300 µl of dichloromethane there were added 180 µl (19.2 µmol) of EDC solution (4 mg/200 µl of CH_2Cl_2) and 36 µl (43.5 µmol) of propylamine solution (10 µl/100 µl of CH_2Cl_2). The reaction mixture was stirred for two days at room temperature. Purification was carried out by means of PLC (CH_2Cl_2/MeOH 90/10) and yielded 1.0 mg (20 %) of **21a**.

ESI MS (1 eV): 885 [M+H^+]; **^{1}H NMR** (CD_3OD, 300 MHz): δ = 2.49 (m, 1 H, Tut2-H), 1.14 (d 3 H, Tut10-H), 3.15 (t, 2 H, Tut11-H), 1.56 (m, 2 H, Tut12-H), 0.96 (t, 3 H, Tut13-H).

*Tubulysin A hexylamide (**21b**): R^1 = OH, R^2 = C_6H_{13} (scheme 6)*

2.8 µl (16.5 µmol) of Hünig's base were dissolved in 200 µl of absolute THF at 0°C and 1.4 µl (11.0 µmol) of isobutyl chloroformate were added. After 5 minutes, 9.3 mg (11.0 µmol) of tubulysin A (**1**) dissolved in 300 µl of absolute THF were added and stirred for a further 40 minutes at 0°C. 1.6 µl (12.1 µmol) of hexylamine and 2.8 µl (16.5 µmol) of Hünig's base were then added to the reaction mixture and stirring was carried out overnight at room temperature. Purification of the crude product was carried out directly by means of PLC (CH_2Cl_2/MeOH 90/10) and yielded, in addition to 6.0 mg (65 %) of **1**, 3.6 mg (35 %) of **21b**.

R_f (CH_2Cl_2/MeOH 90/10): 0.41; **IR** (KBr): $\tilde{\nu}$= 3389 cm^{-1} (m), 2960 (s), 2932 (s), 2872 (w), 1743 (m), 1654 (vs), 1516 (m), 1418 (m), 1228 (s); **UV** (MeOH): λ_{max} (lg ε) = 204 nm (4.62), 226 (4.31), 242 (sh, 4.09), 278 (sh, 3.41); **DCI MS** (120 eV, NH_3): 927 [M+H^+]; **HRMS (DCI):** $C_{49}H_{78}N_6O_9S$: [M+H]$^+$ calc.: 927.5629 (found: 927.5641).

*Tubulysin A benzylamide (**21c**): R^1 = OH, R^2 = $CH_2C_6H_5$ (scheme 6)*

4.5 µl (26.8 µmol) of Hünig's base were dissolved in 200 µl of absolute THF and cooled to 0°C. 2.4 µl (17.9 µmol) of chloroformic acid isobutyl ester were added to the solution and stirring was carried out for 5 minutes. Then, a solution of 10 mg (11.9 µmol) of tubulysin A in 300 µl of absolute THF was added and stirring was carried out at 0°C. After 30 minutes, 1.4 µl (13.1 µmol) of benzylamine and 3 µl (17.9 µmol) of Hünig's base were added and the reaction mixture was stirred overnight at room temperature. The reaction mixture was purified directly by means of PLC (CH_2Cl_2/MeOH 90/10), whereupon 3.5 mg (37 %) of **NT19** were obtained.

R_f (CH_2Cl_2/MeOH 90/10): 0.40; **optical rotation:** $[\alpha]^{20}_D$= +27.2 (c 0.22, methanol); **IR** (KBr): $\tilde{\nu}$= 3383 cm^{-1} (m), 2962 (m), 2935 (m), 2875 (w), 1742 (m), 1661 (vs), 1516 (m), 1420 (w), 1371 (w), 1227 (s), 1094 (w); **UV** (MeOH): λ_{max} (lg ε) = 204 nm (4.62), 224 (sh, 4.24) 246 (sh, 3.94), 277 (sh, 3.24); **DCI MS** (120 eV, NH_3): [M+H^+]; **ESI MS** (1 eV): 932 [M+H)$^+$; **^{1}H NMR** (CD_3OD, 300 MHz): δ = 2.54 (m, 1 H, Tut2-H), 1.64 (m, 1 H, Tut3a-H), 2.03 (m, 1 H, Tut3b-H), 4.24 (m, 1 H, Tut4-H), 2.82 (bd, 2 H, Tut5-H), 7.01 (d, 2 H, Tut7-H), 6.69 (d,

2 H, Tut8-H), 1.17 (d, 3 H, Tut10-H), 4.42 (dd, 2 H, Tut11-H), 7.2-7.4 (m, 5 H, Tut13,14,15-H); **^{13}C NMR** (CD_3OD, 75 MHz): δ = 187.5 (TutC1), 39.1 (TutC2), 40.3 (TutC3), 51.3 (TutC4), 41.3 (TutC5), 130.0 (TutC6), 131.4 (TutC7), 116.2 (TutC8), 157.0 (TutC9), 19.1 (TutC10), 44.2 (TutC11), 140.2 (TutC12), 128.7 (TutC13), 129.5 (TutC14), 128.1 (TutC15).

*Tubulysin derivative **22***

A tubulysin derivative 22 can be obtained by reducing tubulysin 1 using methyl- or ethyl-lithium to form the secondary amine.

*Tubulysin derivative **23***

A tubulysin derivative 23 can be obtained by amidating tubulysin 1 in the presence of EDC in methylene chloride using 1-(2-aminoethyl)-pyrrole-2,5-dione.

*Tubulysin derivative **24***

A tubulysin derivative 24 wherein T = OR^4, $R^4 = SO_3R^6$ and R^6 = H can be obtained by reacting tubulysin 1 with pyridine-SO_3. Analogously, tubulysin 1 can be reacted with phosphoric acid dimethyl ester in the presence of iodine and pyridine in methylene chloride.

*Acetyl-tubulysin A (**25a**): R = iso-C_4H_9, $R^1 = CH_3$ (scheme 7)*

8.9 mg of tubulysin A (**1**) were dissolved in 200 µl of absolute THF, and 8.2 µl (30.6 µmol) of acetyl chloride and 7.1 µmol) of triethylamine were added. The reaction mixture was stirred for 15 minutes at room temperature, 1 ml of water was then added and extraction with ethyl acetate was carried out three times. The combined organic phases were concentrated and dried under a high vacuum. The crude product was purified by means of PLC (CH_2Cl_2/MeOH 90/10), whereupon 5.6 mg (62 %) of **25a** were obtained.

DCI MS (120 eV, NH_3): 886 [M+H$^+$]; **HRMS (DCI):** $C_{45}H_{67}N_5O_{11}S$: [M+H]$^+$ calc.: 886.4636 (found: 886.4701); **^{1}H NMR** (CD_3OD, 300 MHz): δ = 2.58 (m, 1 H, Tut2-H), 1.73 (m, 1 H, Tut3a-H), 2.06 (m, 1 H, Tut3b-H), 4.39 (m, 1 H, Tut4-H), 2.98 (bd, 2 H, Tut5-H), 7.29 (d, 1 H, Tut7-H), 7.00 (d, 1 H, Tut8-H), 1.21 (d, 3 H, Tut10-H), 2.27 (s, 3 H, Tut12-H); **^{13}C NMR** (CD_3OD, 75 MHz): δ = 181.1 (TutC1), 38.7 (TutC2), 39.4 (TutC3), 51.1 (TutC4), 41.2 (TutC5), 137.2 (TutC6), 131.4 (TutC7), 122.5 (TutC8), 150.8 (TutC9), 18.8 (TutC10), 171.2 (TutC11), 20.9 (TutC12).

*Isobutyryl-tubulysin A (**25b**): R = iso-C_4H_9, R^1 = $CH(CH_3)_2$ (scheme 7)*

15.1 mg (17.8 µmol) of tubulysin A (**1**) were dissolved in 400 µl of absolute THF, and 5.6 µl (53.4 µmol) of isobutyric acid chloride and 12.5 µl (89.0 µmol) of triethylamine were added. The reaction mixture was stirred for 30 minutes at room temperature, 2 ml of water were then added and extraction with ethyl acetate was carried out three times. The combined organic phases were dried over sodium sulfate and concentrated. Purification of the crude product was carried out by means of PLC (CH_2Cl_2/MeOH 90/10) and yielded 5.3 mg (32 %) of **NT20**.

R_f (CH_2Cl_2/MeOH 90/10): 0.36; **optical rotation:** $[\alpha]^{20}_D$= +11.5 (c 0.35, methanol); **IR** (KBr): $\tilde{\nu}$= 3392 cm^{-1} (m), 2964 (m), 2936 (m), 2875 (w), 1755 (s), 1668 (vs), 1544 (w), 1508 (w), 1468 (w), 1420 (w), 1371 (w), 1227 (s), 1167 (w); **UV** (MeOH): λ_{max} (lg ε) = 204 nm (4.54), 223 (sh, 4.20); **DCI MS** (120 eV, NH_3): [M+H$^+$]; **ESI MS** (1 eV): 913 [M+H)$^+$; **^{1}H NMR** (CD_3OD, 300 MHz): δ = 2.59 (m, 1 H, Tut2-H), 1.73 (m, 1 H, Tut3a-H), 2.07 (m, 1 H, Tut3b-H), 4.39 (m, 1 H, Tut4-H), 2.98 (bd, 2 H, Tut5-H), 6.99 (d, 2 H, Tut7-H), 7.30 (d, 2 H, Tut8-H), 1.22 (d, 3 H, Tut10-H), 2.82 (d, 1 H, Tut12-H),1.31 (d, 6 H, Tut13,14-H); **^{13}C NMR** (CD_3OD, 75 MHz): δ = 181.1 (TutC1), 38.7 (TutC2), 39.4 (TutC3), 51.1 (TutC4), 41.2 (TutC5), 137.2 (TutC6), 131.4 (TutC7), 122.3 (TutC8), 150.8 (TutC9), 18.8 (TutC10), 177.2 (TutC11), 35.3 (TutC12), 19.2 (TutC13,14).

*Tubulysin A allyl ether allyl ester (**26a**): R = iso-C_4H_9, R^1 = CH_2CHCH_2 (scheme 7)*

To 6.0 mg (7.1 µmol) of tubulysin A (**1**), dissolved in 300 µl of dichloromethane/diethyl ether (1/1), there were added 6.2 µl (71.2 µmol) of allyl bromide and 6.6 mg (28.5 µmol) of silver(I) oxide and stirring was carried out overnight at room temperature. Filtration over Celite was then performed. The residue was washed with dichloromethane and the combined organic phases were concentrated. Purification of the crude product was carried out by means of PLC (CH_2Cl_2/MeOH 90/10) and yielded 2.9 mg (44 %) of **26a**.

ESI MS (1 eV): 924 $[M+H)^+$; **^{1}H NMR** (CD_3OD, 400 MHz): δ = 7.17 (d, 2 H, Tut7-H), 6.86 (d, 2 H, Tut8-H), 1.22 (d, 3 H, Tut10-H), 4.5-4.6 (m, 4 H, Tut11-H, Tut14-H), 5.93 (m, 1 H, Tut12-H), 5.1-5.5 (m, 4 H, Tut13-H, Tut16-H), 6.07 (m, 1 H, Tut15-H).

*Tubulysin A methyl ether methyl ester (**26b**): R = iso-C_4H_9, R^1 = CH_3 (scheme 7)*

21.7 mg (25.7 µmol) of tubulysin A (**1**) were dissolved in 200 µl of methanol; ethereal diazomethane solution was added at room temperature on three occasions spaced 15 minutes apart and stirring was carried out overnight. The reaction mixture was then dried. Purification was carried out by means of PLC (CH_2Cl_2/MeOH 90/10) and yielded 10.3 mg (46 %) of **26** and 5.0 mg (23 %) of tubulysin A methyl ester (**20a**).

DCI MS (120 eV, NH_3): 872 $[M+H^+]$; **HRMS (DCI):** $C_{45}H_{69}N_5O_{10}S$: $[M+H]^+$ calc.: 872.4843 (found: 872.4818); **^{1}H NMR** (CD_3OD, 400 MHz): δ = 2.6 (m, 1 H, Tut2-H), 2.86 (d, 2 H, Tut5-H), 7.17 (d, 2 H, Tut7-H), 6.85 (d, 2 H, Tut8-H), 1.19 (d, 3 H, Tut10-H), 3.65 (s, 3 H, Tut11-H), 3.78 (s, 3 H, Tut12-H)

*Tubulysin A methyl ether (**27a**): R = iso-C_4H_9, R^1 = CH_3 (scheme 7)*

1.6 mg (1.8 µmol) of **26a** (R^1 = CH_3) were dissolved in 50 µl of DMSO, and 700 µl of phosphate buffer (20mM KH_2PO_4, pH = 7.3) were added. The reaction batch was placed for 5 minutes in an ultrasonic bath, 72 µl of pig liver esterase (Boehringer-Mannheim) were then added and stirring was carried out for 4 hours at 36°C. For isolation of the product,

extraction was carried out with ethyl acetate three times, and the combined organic phases were dried. Purification of the crude product was carried out by means of PLC (CH_2Cl_2/MeOH 90/10) and yielded 0.5 mg (32 %) of **27a**.

DCI MS (120 eV, NH_3): 858 [M+H$^+$]; **HRMS (DCI):** $C_{44}H_{67}N_5O_{10}S$: [M+H]$^+$ calc.: 857.4687 (found: 858.4740); **^{1}H NMR** (CD_3OD, 300 MHz): δ = 2.8 (m, 2 H, Tut5-H), 7.19 (d, 2 H, Tut7-H), 6.84 (d, 2 H, Tut8-H), 3.78 (s, 3 H, Tut11-H).

*Iodination of tubulysin A (**28a, 29a**): R = iso-C_4H_9, Hal = I (scheme 8)*

11.0 mg (13.1 µmol) of tubulysin A (**1**) were dissolved in 200 µl of methanol, and 13.0 µl of iodine monochloride solution (13.1 µmol) were added. The reaction mixture was stirred for 15 minutes at room temperature and then purified directly by means of PLC (CH_2Cl_2/MeOH 90/10). 3.1 mg (25 %) of **28a** and 3.9 mg (27 %) of **29a** were obtained in the process.

28a:

ESI MS (1 eV): 970 [M+H]$^+$; **^{1}H NMR** (CD_3OD, 300 MHz): δ = 2.58 (m, 1 H, Tut2-H), 1.71 (m, 1-H, Tut3a-H), 2.05 (m, 1-H, Tut3b-H), 4.28 (m, 1-H, Tut4-H), 2.82 (m, 2 H, Tut5-H), 7.10 (dd, 2 H, Tut7-H), 6.75 (d, 2 H, Tut8-H), 7.55 (d, 1 H, Tut12-H); **^{13}C NMR** (CD_3OD, 75 MHz): δ = 181.2 (TutC1), 38.7 (TutC2), 39.5 (TutC3), 51.4 (TutC4), 40.6 (TutC5), 132.6 (TutC6), 131.5 (TutC7), 115.6 (TutC8), 156.5 (TutC9), 18.8 (TutC10), 84.4 (TutC11), 141.2 (TutC12).

29a:

ESI MS (1 eV): 1096 [M+H]$^+$; **^{1}H NMR** (CD_3OD, 600 MHz): δ = 2.58 (m, 1 H, Tut2-H), 1.73 (m, 1 H, Tut3a-H), 2.05 (m, 1 H, Tut3b-H), 4.25 (m, 1 H, Tut4-H), 2.75 (dd, 1 H, Tut5a-H), 2.86 (dd, 1 H, Tut5b-H), 7.61 (s, 2 H, Tut7,12-H); **^{13}C NMR** (CD_3OD, 75 MHz): δ = 181.1 (TutC1), 38.6 (TutC2), 39.6 (TutC3), 51.5 (TutC4), 40.1 (TutC5), 135.8 (TutC6), 141.5 (TutC7,12), 85.1 (TutC8,11), 155.2 (TutC9), 18.8 (TutC10).

*Nitro-tubulysin A (**30a**): R = iso-C_4H_9 (scheme 8)*

To a solution of 12.5 mg (14.8 µmol) of tubulysin A (**1**) in 400 µl of ethanol there were added 100 µl of glacial acetic acid and 20.5 mg (296.6 µmol) of sodium nitrite, dissolved in 100 µl of water. The reaction mixture was stirred for two days at room temperature and was then dried under a high vacuum. The crude product was purified by means of PLC (CH_2Cl_2/MeOH 90/10), whereupon 9.8 mg (74 %) of **30** were obtained.

IR (KBr): $\tilde{\nu}$= 3411 cm^{-1} (m), 2962 (m), 2932 (m), 2873 (w), 1741 (s), 1666 (vs), 1539 (s), 1492 (w), 1424 (w), 1370 (w), 1223 (s); **UV** (MeOH): λ_{max} (lg ε) = 205 nm (4.56), 216 (sh, 4.42), 234 (sh, 4.19), 274 (3.77), 360 (3.43); **DCI MS** (120 eV, NH_3): 889 [$M+H^+$]; **^{1}H NMR** (CD_3OD, 400 MHz): δ = 2.60 (m, 1 H, Tut2-H), 1.74 (m, 1 H, Tut3a-H), 2.09 (m, 1 H, Tut3b-H), 4.37 (ddd, 2 H, Tut4-H), 2.91 (dd, 1 H, Tut5a-H), 3.01 (dd, 1 H, Tut5b-H), 7.56 (dd, 1 H, Tut7-H), 7.07 (d, 1 H, Tut8-H), 1.27 (d, 3 H, Tut10-H), 7.97 (d, 1 H, Tut12-H); **^{13}C NMR** (CD_3OD, 400 MHz): δ = 180.7 (TutC1), 38.5 (TutC2), 39.6 (TutC3), 51.0 (TutC4), 40.7 (TutC5), 132.0 (TutC6), 139.4 (TutC7), 120.8 (TutC8), 154.2 (TutC9), 18.8 (TutC10), 135.3 (TutC11), 126.5 (TutC12).

*Tubulysin derivatives **31 and 32***

A tubulysin derivative 31 can be obtained by catalytically reducing nitro-tubulysin A (30a) in ethanol using elemental hydrogen together with a Pd/C catalyst.

The tubulysin derivative 31 obtained can be acylated to form a tubulysin derivative 32 using acetic anhydride.

*Tubulysin A N-oxide (**33a**): R = iso-C_4H_9 (scheme 9)*

9.9 mg (11.7 µmol) of tubulysin A (**1**) were dissolved in 200 µl of dichloromethane; 290 µl (11.7 µmol) of *m*-CPBA solution (10 mg/ml of dichloromethane) were added and stirring was carried out at room temperature for 30 minutes. After the reaction mixture was

reduced, purification was carried out directly by means of PLC (CH_2Cl_2/methanol 85/15), whereupon 5.2 mg (52 %) of **33a** were obtained.

ESI MS (1 eV): 860 $[M+H]^+$.

*Tubulysin derivative **34***

A tubulysin derivative 34 can be obtained by treating tubulysin A N-oxide (33a) with acetic anhydride at about 75°C.

Abbreviations

Abbreviation	Name
C_5Cl_5NF-triflate	*N*-fluoropentachloropyridinium triflate
CH_3CN	acetonitrile
DAST	(diethylamino)sulfur trifluoride
DMAP	dimethylaminopyridine
EDC	*N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide
ICl	iodine monochloride
m-CPBA	*meta*-chloroperbenzoic acid
Me_3SiCl	trimethylchlorosilane
$NaCNBH_3$	sodium cyanoborohydride
NBS	*N*-bromosuccinimide
NMO	*N*-methyl-morpholine *N*-oxide
p-CH_3-$C_6H_4SO_2OH$	para-toluenesulfonic acid
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TPAP	*tetra*-propylammonium perruthenate